

VIRGINIA POLYTECHNIC INSTITUTE
AGRICULTURAL BRANCH LIBRARY
BLACKSBURG, VIRGINIA

Thermal Death-Time Studies of Coliform Bacteria in Milk

J. C. Olson, Jr., H. Macy, and H. O. Halvorson

Division of Dairy Husbandry and Department of Bacteriology and Immunology



University of Minnesota
Agricultural Experiment Station

Page
57
57
58
58
44
50
50
10
57
57
9
9
10
10
9
31
31
31
61
9
44
17
56
56
14
31
39
9
59
44
44
45
45
14
60
11
15
14
14
12
12
13
22
51
31
46
46
19
51
47
34
34
34
20
46
24, 27
32
32

CONTENTS

	Page
Introduction	3
Materials and methods	5
Isolation and characterization of cultures	5
Methods for determining heat resistance	5
Experimental procedure	7
Variation in heat resistance among coliform cultures	7
Survival after heat treatment of cultures incubated at 37° C. for 16 and 24 hours before exposure	8
Effects of previous temperature of incubation on heat resistance	12
Effects of population on the survival of cultures after various heat treatments	14
Delayed growth after heat treatment	15
Discussion	15
Conclusions	17
Literature cited	19

Thermal Death-Time Studies of Coliform Bacteria in Milk

J. C. Olson, Jr., H. Macy, and H. O. Halvorson²

INTRODUCTION

OUR UNDERSTANDING of the principles involved in the destruction of bacteria by agents unfavorable to them stems from the observations of Kronig and Paul (26) who studied the destructive effects of various chemical compounds on anthrax spores. Kronig and Paul, and later Chick (11), who included heat as an agent of destruction, observed that the organisms were destroyed in an orderly manner with time. These observations have been extended by many others (7, 9, 17, 45, 47) and have led to the conclusion that the order of death of bacterial vegetative cells and spores by heat is logarithmic. The greatest amount of information concerning the thermal destruction of bacteria has been derived from studies using spore-forming species. Detailed reviews of these studies have been reported (28, 33, 36).

Near the beginning of the extensive work on the effectiveness of thermal processes in destroying spore-forming bacteria in canned foods, Bigelow, Bostart, Richardson, and Ball (10) developed a method of computing the time required to obtain sterility in cans of food. Later, Ball (2, 3), in an extension of this work, introduced the symbol "F" to indicate the thermal death-time of any organism at 250° F. and "z" to characterize the slope of its thermal death-time curve. More specifically, z represented the increase in degrees Fahrenheit which was necessary to reduce the thermal death-time tenfold. The "F₀" value, which often is used, was intended originally (3) to express the F value when z equaled 18. Thermal

death-time may be defined as the time required to kill an organism at a given temperature and in a known environment. Thus, when the values of F and z pertaining to any organism under specific conditions are known, the thermal death-time at any other temperature may be obtained.

Others have suggested symbols similar to F for use in calculating thermal processing times for foods not requiring heat treatments as severe as those to which the symbol F was applied. For example, Gross and Schaub (20) suggested the symbol "F'" to represent the thermal death-time of any organism at 150° F. Baselt (6) introduced the symbol "Z" to characterize the slope of the bacterial rate of destruction curve.

¹ Taken from data presented in a thesis submitted to the graduate faculty of the University of Minnesota by J. C. Olson, Jr., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
² Associate professor, Division of Dairy Husbandry, University of Minnesota; director, University of Minnesota Agricultural Experiment Station; and head of the Department of Bacteriology, University of Illinois, Urbana, Illinois, respectively.
 The authors acknowledge funds for research contributed by the Twin City Quality Control Committee and the Quality Control Laboratory.

The value of Z is expressed as minutes, while that for z is expressed as degrees Fahrenheit. Recent reports indicate some confusion as to the meaning of these two terms. Gillespy (19) introduced the symbol "D" to represent the slope of the rate of destruction curve. Recently, Schmidt (34) suggested the replacement of the symbol Z with the symbol D to avoid confusion with the lower case z.

Ball (4) has discussed the application of thermal death-time curves in connection with heat treatments to which milk and other dairy products may be subjected. With few exceptions the data on the thermal destruction of non-spore-forming bacteria are of such nature that thermal death-time curves for the organisms studied cannot be constructed. Investigations that partially led to the establishment of present time and temperature relationships for pasteurization of milk provided considerable knowledge regarding thermal death-times for certain pathogens likely to be present in milk (27, 29, 30, 31). Approximate thermal death-time curves for the organisms studied may be constructed from certain of the data. Other investigators (1, 7, 21) have reported thermal death-time curves for non-spore-forming organisms suspended in products other than dairy products. When these organisms are grown and heated in milk, the position and slope of their thermal death-time curves may be quite different from those reported. The nature of the medium in which organisms are cultured or of the medium in which they may be suspended during heat treatment may greatly influence the thermal resistance of the organisms.

Slatter and Halvorson (37) studied a large number of lactobacilli and found wide variations in heat resistance among different cultures which had been grown in milk and subsequently suspended in milk during heat treatment. The z values for thermal death-

time curves of selected cultures ranged from 8.0 to 13.0. Holland and Dahlberg (24) obtained a thermal death-time curve for *Escherichia coli* based on 99.99 per cent destruction of the culture suspended in milk during heat treatment. Their data indicated a z value of approximately 9.5. Similar curves for three cultures of *E. coli* were reported by Gilcreas and O'Brien (18). A z value of approximately 9.2 for each curve was indicated by their data. Speck (38) reported two thermal death-time curves (99.99 per cent destruction) for a culture of *Micrococcus freudenreichii*, one heated in milk and the other in ice cream mix. From the data presented, a z value of approximately 8.3 was indicated for each curve.

As nearly as can be determined from the literature, z values for thermal death-time curves of non-spore-forming bacteria ranged from 8.0 to 20.0. It is significant that data which are most adaptable for analysis indicate that values nearer the lower figure were most common. In most of the studies which have been reported involving the heat resistance of non-spore-forming bacteria, only a single temperature of exposure and a single interval of time at a given temperature were used. Little attention was given to the concentration of organisms subjected to heat treatment, to the lethality during the period necessary to bring the suspension to the temperature of exposure, or to the lethality during the cooling time from that temperature after the stated exposure time. Age of the culture, stage of growth, and natures of the growth medium and the suspension medium, all of which are factors affecting thermal resistance, often were ignored or not stated.

Except for data reported by Holland and Dahlberg (24) and by Gilcreas and O'Brien (18), none have been found that can be used to construct thermal death-time curves for coliform bacteria which have been heated in milk.

The varied heat treatments to which milk and other dairy products are subjected during processing indicate the need for information regarding the effect of such heat treatments on bacteria which may be present in the products.

The objectives of this investigation were to obtain information regarding the following: (1) the variation in heat resistance among a rather diverse group of bacteria; (2) the variation in heat resistance of a pure culture upon repeated trials under similar conditions of manipulation; (3) the effects of various growth conditions on position and slope of thermal death-time curves of representative cultures; (4) the varia-

tion in slope of thermal death-time curves which might occur on repeated trials under similar conditions of culture treatment. A fifth objective was to obtain data bearing on the theoretical explanation given by Ball (4) for the differences observed in bacterial destruction between high-temperature, short-time and low-temperature, holding methods of pasteurization.

Coliform bacteria were chosen to be used in this study. The diversity of these bacteria, their wide distribution, and their importance to the dairy industry would seem to indicate that this group is suitable for a study of this nature.

MATERIALS AND METHODS

Isolation and Characterization of Cultures

Coliform cultures were isolated from a variety of sources including pasteurized milk and cream, raw milk and cream, chocolate milk, ice cream, cottage cheese, butter, smoked cheese, water, and human feces.

Morphological and biochemical characteristics of purified cultures were determined, for the most part, in accordance with the procedures outlined in the *Manual of Methods for Pure Culture Study of Bacteria* (13). Motility was determined in semisolid medium using the procedure of Tittler and Sandholzer (42). The Voges-Proskauer reaction was determined by the procedure of Barritt (5) modified by incubation at 30° C. for 24 to 48 hours as suggested by Vaughn, Mitchell, and Levine (44). The methyl red reaction was determined after incubation at 30° C. for five days as recommended by Clark and Lubs (12). Citrate utilization was determined using the medium of Koser (25) with incubation at 37° C. for three days. Hydrogen sulfide production was determined using the

medium recommended by Tittler and Sandholzer (43) with incubation at 37° C. for six days. Liquefaction of gelatin was determined after incubation at 37° C. for four weeks in nutrient broth containing 10 per cent gelatin.

The coliform cultures were classified according to three schemes, Parr (32), Bergey et al. (8), and Wilson (49).

Methods for Determining Heat Resistance

Stock cultures of all organisms used in heat-resistance trials were carried in sterilized, fresh litmus milk. These cultures were transferred at two-week intervals. After transfer, the cultures were incubated at 37° C. for 24 hours and then stored at 5° C. until the next transfer was made.

The preparation of cultures used in heat-resistance trials, unless otherwise stated under presentation of data, was as follows. Each culture was grown through two transfers in sterilized, fresh litmus milk at the desired temperature and for the desired period of time. The culture representing the

second transfer was used to inoculate 100 milliliters of sterilized, fresh skim milk which did not contain litmus. This milk was placed in six-ounce, screw-cap, medicinal ovals. Each bottle contained 30 to 50 small glass beads.

The amount of inoculum was controlled by use of a standardized loop, so that the inoculated skim milk contained approximately 50,000 to 100,000 viable cells per milliliter as determined by plate count.

The 100-milliliter amount of skim milk was incubated at the temperature and for the time desired. After incubation the culture was shaken vigorously 50 to 75 times to assist in breaking up clumps of bacteria. Following shaking, 0.5 milliliter of the culture was added to 100 milliliters of cold (5° C.), sterile litmus milk contained in six-ounce medicinal ovals. The bottle was shaken 50 to 75 times, after which one milliliter was transferred to each of the required 100-milliliter quantities of cold litmus milk. This was the final dilution, and the suspension which resulted after shaking was the one heated. A plate count of this suspension was made before heating. Counts of suspensions prepared in this manner were quite regularly between 35,000 and 65,000 per milliliter.

Approximately 2.25 milliliters of the final suspension were transferred to each of the desired number of 10- x 75-millimeter test tubes, Kimble-brand No. 45050. These tubes were sealed by flame. Appropriate exposures were made by submerging the tubes in a constant-temperature water bath. Usually, ten tubes were used for each exposure time at any one temperature. In preliminary trials designed to obtain general heat-resistance levels, fewer tubes for each exposure time were used. Occasionally, a tube was broken during the procedure. In the tables which follow, the total number of tubes exposed for the time at the temperature indicated was the com-

bined total of tubes showing growth and no growth. Time intervals were measured by a stop watch. After exposure the tubes were cooled immediately in a water bath held at 60° to 65° F.

Following heat treatment and cooling, the tubes were placed in racks and incubated at 35° to 37° C. The tubes were observed for signs of growth approximately every other day, with the final reading being made after ten days. The thermal death-time at any temperature was taken to be the time of exposure which yielded the first series of tubes without detectable growth in all tubes.

The constant-temperature water baths used in this study were the Precision, cylindrical all-metal type. Maximum temperature variation was $\pm 0.2^\circ$ F.

The device described by Slatter and Halvorson (37) was used to determine the total time required to bring the contents of the tubes to the temperature of exposure. This consisted of a skim milk thermometer attached to a meter stick. The skim milk thermometer was made by attaching a 7/15-inch, ground-glass joint to the end of a 30-inch capillary. The companion joint was attached to one of the Kimble tubes. Small glass hooks were sealed on both pieces to provide anchors for two springs which maintained a complete seal between the capillary and the tube. The ground-glass joint was a modification of the milk thermometer used by Slatter and Halvorson (37). This thermometer was calibrated against a mercury thermometer by immersion in a constant-temperature water bath at temperatures differing by 2° F. and extending over the heating and cooling range used in these studies. The heating lag to any temperature desired was obtained by placing the milk thermometer in the bath and recording the reading on the meter stick at 15-second intervals. The time of heating

Table 1. Time Correction for Heating Skim Milk in Kimble Tubes

Time of heating contents	Temperatures of tube contents	Time equivalent (minutes) at 134° F. when z equaled			Temperatures of tube contents	Time equivalent (minutes) at 140° F. when z equaled			Temperatures of tube contents	Time equivalent (minutes) at 148° F. when z equaled		
		10.5	11.5	12.5		10.5	11.5	12.5		10.5	11.5	12.5
minutes	°F.				°F.				°F.			
0	70.0				70.0				70.0			
0.25	105.5				112.0				116.5			
0.50	122.0				129.0				136.9			
0.75	129.0	0.039	0.045	0.053	135.5	0.047	0.052	0.060	143.5	0.042	0.048	0.054
1.00	131.5	0.110	0.115	0.125	138.0	0.125	0.130	0.135	146.0	0.120	0.127	0.135
1.25	132.5	0.160	0.165	0.170	139.0	0.180	0.185	0.190	147.9	0.180	0.185	0.188
1.50	133.5	0.200	0.205	0.210	139.5	0.213	0.215	0.218	147.5	0.215	0.218	0.220
1.75	134.0	0.235	0.238	0.240	140.0	0.240	0.240	0.240	148.9	0.235	0.240	0.240
Total		0.744	0.768	0.798		0.805	0.822	0.843		0.792	0.818	0.837
Therefore:					Time correction (minutes) at 140° F.:				Time correction (minutes) at 148° F.:			
1.006	(1.75 - 0.744) when z = 10.5				0.945	(1.75 - 0.805) when z = 10.5			0.956	(1.75 - 0.792) when z = 10.5		
0.982	(1.75 - 0.768) when z = 11.5				0.928	(1.75 - 0.822) when z = 11.5			0.932	(1.75 - 0.818) when z = 11.5		
0.952	(1.75 - 0.798) when z = 12.5				0.907	(1.75 - 0.843) when z = 12.5			0.913	(1.75 - 0.837) when z = 12.5		

and cooling was converted to an equivalent time at the temperature of the constant-temperature bath according to the method of Halvorson (23). The data obtained in determinations of time corrections for exposures at 134°, 140°, and 148° F. are shown in table 1.

Time corrections were determined using z values of 10.5, 11.5, and 12.5. The data presented show that in all instances the contents of the tubes required a total of 1.75 minutes to reach the exposure temperature. Furthermore, it may be observed in all instances that 1.75 minutes, when converted to an equivalent time at the exposure temperature used, became approximately 0.8 minute. Actually, the equivalent periods ranged from 0.744-0.843 minute depending upon the temperatures of exposure and the z values

which were used in making the calculations. When these values were subtracted from 1.75 minutes, the remainders closely approximated one minute. When intervals of exposure at temperatures other than those indicated in table 1 were used, the corrections for heating lags were similar; therefore, when thermal death-time curves were plotted, one minute was subtracted from all time intervals for which tubes were exposed.

After the tubes had been exposed for the desired period of time, they were immersed in a water bath at 60° to 65° F. Cooling occurred with such rapidity that the time correction for the cooling lag was insignificant in relation to the total exposure time. This observation was similar to that of Slatter (36).

EXPERIMENTAL PROCEDURE

Variation in Heat Resistance among Coliform Cultures

When possible, each of the 139 coliform cultures was classified according to the three schemes mentioned previously. Since the essential criteria of

Parr's scheme of grouping consist of the "Invic" reactions, this was the only scheme by which all cultures could be grouped by the observations which were made. Eleven of the 16 possible "Invic" types were found among the cultures.

Table 2. Distribution of Coliform Cultures which Survived Various Periods of Exposure at 135° F.

Classification*	Cultures surviving:											
	Less than 5 minutes		5 to 10 minutes		10 to 20 minutes		20 to 40 minutes		40 to 60 minutes		60 or more minutes	
	number	per cent	number	per cent	number	per cent	number	per cent	number	per cent	number	per cent
<i>A. aerogenes</i> section	10	38.5	14	48.3	24	60	9	52.9	1	6.3	0	0
<i>E. coli</i> section	1	3.8	2	6.9	10	25	5	29.4	15	93.7	10	96.9
Intermediate section	15	57.7	13	44.8	6	15	3	17.6	0	0	1	9.1
Total	26	100.0	29	100.0	40	100.0	17	99.9	16	100.0	11	100.0

* According to Parr (32).

Each culture was tested by procedures given above to determine its approximate thermal death-time at 135° F. Exposures of 5, 10, 20, 40, and 60 minutes were made, using two tubes of litmus milk suspension for each interval of exposure. The distribution, according to heat resistance at 135° F., of all cultures within the three coliform sections of Parr is shown in table 2.

These data have been reported in greater detail (28). It may be observed that the greatest percentage (57.7 per cent) of the most heat-sensitive cultures was classed in the intermediate section; 38.5 per cent belonged to the *Aerobacter aerogenes* section. Cultures in the "5-10" minute group were fairly evenly divided between the *A. aerogenes* and the intermediate sections, although two cultures in this group belonged to the *E. coli* section. In the "10-20" group, cultures in the *A. aerogenes* section predominated (60 per cent), while the number of cultures in the intermediate section decreased to 15 per cent and the number of those in the *E. coli* section increased to 25 per cent. The distribution in the "20-40" group was not changed appreciably from that noted in the "10-20" group. A marked change in distribution occurred among the cultures in the "40-60" group. The *E. coli* section represented 93.7 per cent of the cultures in this group, one culture belonged to the *A. aerogenes* section, and one to the

intermediate section. The distribution remained essentially the same for the cultures which survived 60 minutes, although one culture was classed in the intermediate section. No culture representative of the *A. aerogenes* section survived the 60-minute exposure period.

A wide range of heat resistance within the coliform group is evident from the data presented. Furthermore, the cultures conformed to a rather regular pattern, since those representative of the intermediate section appeared to be largely the most heat sensitive, while those representative of the *A. aerogenes* section occupied a somewhat more intermediate position in that regard. The two most heat-resistant groups consisted almost entirely of cultures representative of the *E. coli* section.

Survival after Heat Treatment of Cultures Incubated at 37° C. for 16 and 24 Hours before Exposure

For the studies which involved the determination of thermal death-time curves, a culture was desired which had a thermal death-time at 143° F. of about 20 to 25 minutes. Consequently, two of the most heat-resistant cultures of *E. coli* were selected for use. These were designated as cultures 02 and 07. An attempt was made to determine the extent of variation in position and

Table 3. Survival of Culture 02 after Various Heat Treatments (Culture Incubated at 37° C. for 16 Hours before Exposure)

Number of tubes showing growth after exposure for time at temperature indicated (+ growth; — no growth)											
Trial 1*											
minutes at 135° F.	number	minutes at 138° F.	number	minutes at 140° F.	number	minutes at 141.8° F.	number				
		number		number		number		number	per cent	per cent	per cent
30	3+ 2—	47	4+ 1—	28	5+	14	5+				
90	5—	52	5—	31	5+	16	5+				
105											
Trial 2*											
95	8+ 2—	52	4+ 6—	36	1+ 9—	21.5	1+ 9—				
105	10—	55	10—	40	10—	24	10—				
Trial 3*											
61	10+	21	10+	9	9+ 1—	3.5	10+				
65	8+ 2—	23	5+ 5—	10	3+ 7—	4.0	4+ 6—				
Trial 4*											
61	10+	23	8+ 2—	10	2+ 8—	4.0	1+ 9—				
65	7+ 3—	25	7+ 3—	11	9—	4.5	10—				
Trial 5*											
55	10—	21	10—	8.25	1+ 9—	3.5	2+ 8—				
58	10—	23	10—	9.0	10—	4.0	10—				
Trial 6*											
75	1+ 9—	28	4+ 6—	16.5	2+ 8—	6.0	2+ 8—				
82	10—	32	10—	18.5	10—	6.75	10—				

* The following indicates population of suspensions used in trials indicated: Trial 1 = 26,000 per milliliter; Trial 2 = 50,000 per milliliter; Trial 3 = 87,000 per milliliter; Trial 4 = 62,000 per milliliter; Trial 5 = 57,000 per milliliter; Trial 6 = 77,000 per milliliter.

slope of thermal death-time curves which might occur on repeated trials under similar conditions of culture treatment. The cultures were incubated at 37° C. for 16 hours before heat treatment. From five to ten exposure times were used for each temperature. Pertinent portions of the data obtained from six trials using culture 02 are presented in table 3. Trial 1 was exploratory and was designed to obtain the general level of heat resistance of the culture. The results of this trial were used as a guide in selecting an adequate range of exposure times at various temperatures for use in subsequent trials. In this connection, at least two exposure times at each temperature which would yield at least one or more positive tubes, and two exposure times at each temperature which would result in all negative tubes were desired. It may be observed that end points were reached in trials 2 and 6. These data were used to plot the thermal death-time curves shown in figures 1 and 2.

It is evident from the data presented that the thermal death-time of culture 02 varied considerably on repeated trials. For example, in trials 3 and 4 the culture survived an exposure of 65 minutes at 138° F., while in trials 2 and 5 the survival time was less than 55 minutes at the same temperature. Variations at other temperatures were relatively the same. The position and slope of the two thermal death-time curves are essentially the same; the z value of one being 10.5, the other 10.8. The curves indicate that under the conditions of trials 2 and 6, the culture would be killed by the low-temperature holding method of pasteurization.

Variations in thermal death-time data which were obtained for culture 07 were similar to those obtained with culture 02. Data which were satisfactory for plotting a thermal death-time curve for this culture, however, were not obtained because end points were not obtained at four different temperatures during any single trial.

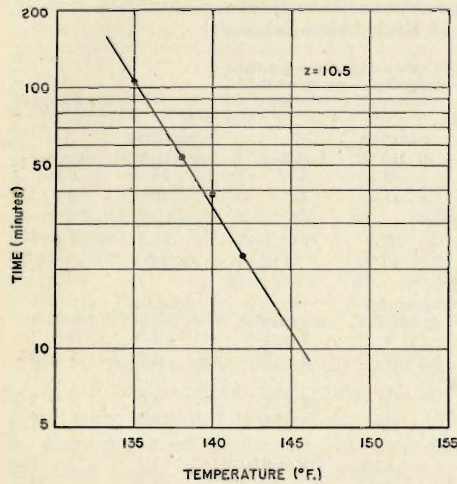


FIG. 1. Thermal death-time curve: Culture 02 after growth in skim milk for 16 hours at 37° C. (Trial 2, population approximately 50,000 per milliliter)

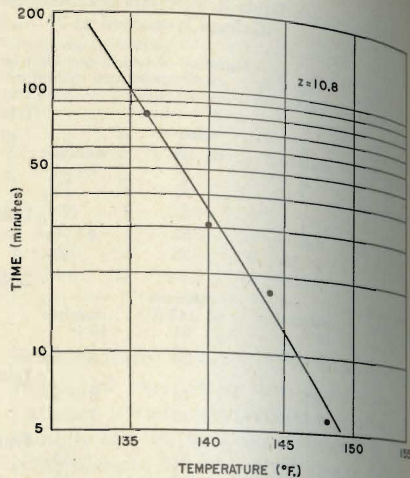


FIG. 2. Thermal death-time curve: Culture 02 after growth in skim milk for 16 hours at 37° C. (Trial 6, population approximately 50,000 per milliliter)

To determine whether or not the incubation period which was used might have contributed to the considerable variation in heat resistance observed, thermal death-time trials were made, using culture 02 incubated at 37° C. for 16, 20, 26, and 28 hours. At the end of each of these periods, the culture was in the maximum stationary growth phase.

No definite evidence was obtained that the heat resistance of cultures

compared at various stages of growth was significantly different, although the results of trials using 24-hour cultures appeared to be more uniform. In view of this, three attempts were made to obtain thermal death-time curves for culture 02 incubated at 37° C. for 24 hours before heat treatment. A portion of the data from these trials is presented in table 4. It may be observed that the heat resistance of the culture remained approximately the same for

Table 4. Survival of Culture 02 after Various Heat Treatments (Culture Incubated at 37° C. for 24 Hours before Exposure)

Number of tubes showing growth after exposure for time and at temperature indicated (+ growth; — no growth)							
minutes at 136° F.		minutes at 140° F.		minutes at 144° F.		minutes at 148° F.	
90	8+ 2—	44	5+ 5—	18	1+ 9—	7.5	7+ 3—
100	10—	49	10—	20	10—	8.5	10—
Trial 1*							
100	5+ 5—	40	5+ 5—	18	3+ 7—	9.5	1+ 9—
110	10—	44	10—	20	10—	10.5	10—
Trial 2*							
120	7+ 2—	59	7+ 3—	24	1+ 9—	10.5	1+ 9—
135	3+ 7—	64	3+ 7—	26	1+ 9—	11	1+ 9—
Trial 3*							

* The following indicates population of suspensions used: Trial 1 = 54,000 per milliliter; Trial 2 = 64,000 per milliliter; Trial 3 = 51,000 per milliliter.

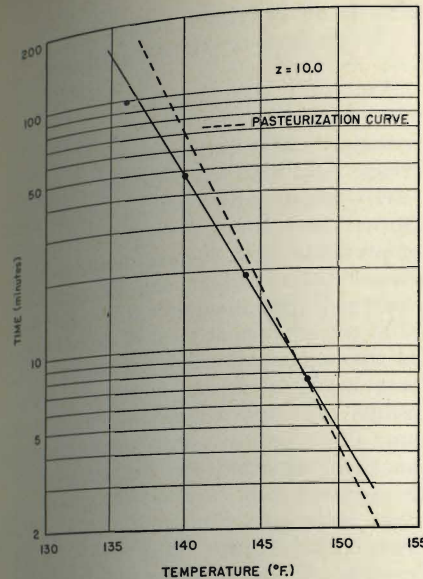


FIG. 3. Thermal death-time curve: Culture 02 after growth in skim milk for 24 hours at 37° C. (Trial 1, population approximately 50,000 per milliliter)

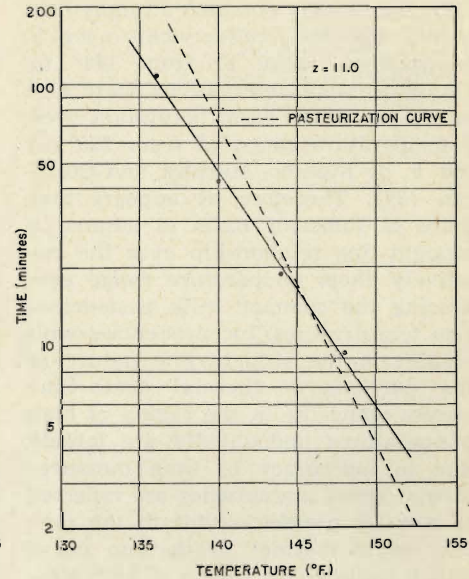


FIG. 4. Thermal death-time curve: Culture 02 after growth in skim milk for 24 hours at 37° C. (Trial 2, population approximately 50,000 per milliliter)

trials 1 and 2. In trial 3, the culture showed a marked increase in heat resistance in spite of the fact that methods of handling the culture were the same as used previously. For example, from trial 2, a thermal death-time of 43 minutes at 140° F. is indicated; from trial 3, a thermal death-time in excess of 63 minutes at 140° F. is indicated. The data from trials 1 and 2 were used to plot the thermal death-time curves shown in figures 3 and 4. The z values were 10 and 11. They were not greatly different from those indicated in figures 1 and 2.

In figures 3 and 4 the "pasteurization curve" also is shown. This curve was plotted by connecting two points representing the two minimum pasteurization time-temperature relationships which are commonly used, 143° F. for 30 minutes and 160° F. for 15 seconds. If time and temperature relationships

represented by an extension of the thermal death-time curves beyond the last determined point and in the direction of higher temperature would hold, it is evident that each culture would have survived heat treatments represented by points on the pasteurization curve which are below the points of intersection of the respective thermal death-time curve and the pasteurization curve. Thus, the thermal death-time curves indicate that under conditions of these trials the cultures would be killed at 143° F. for 30 minutes but would survive 160° F. for 15 seconds.

Evidence exists in justification of extending the thermal death-time curves. Holland and Dahlberg (24) obtained a straight-line curve for *E. coli* which extended from approximately 140° to 165° F. Three similar curves extending from approximately 144° to 167° F. were obtained by Gilcreas and O'Brien

(18). Speck (38) obtained straight-line curves for *M. freudenreichii* over a temperature range of from 140° to 170° F. Similar observations were obtained for *Clostridium botulinum* over a temperature range of from 220° to 260° F. by Stumbo, Murphy, and Cochran (39). Therefore, it appears that there is sufficient basis to assume a straight-line relationship over the relatively short temperature range embracing the common milk pasteurization temperatures. Inconsistencies with respect to the straight-line nature of the portions of thermal death-time curves which lie in the region of high temperatures undoubtedly are largely due to inaccuracy of time measurements. These inaccuracies are reflected to a much greater extent in the portion of a thermal death-time curve which includes the region of high temperatures and short times than in the portion which includes low temperatures and long exposure periods.

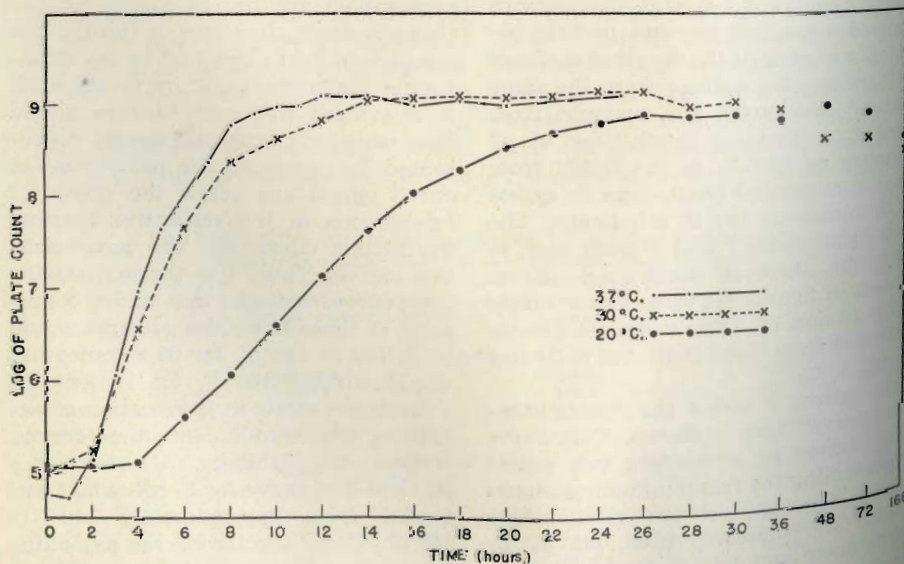


FIG. 5. Growth curves for Culture 02 in skim milk

Effects of Previous Temperature of Incubation on Heat Resistance

Weil (46) was probably the first to observe that the ability of a culture to withstand heat increased with an increase in growth temperature. Williams (48) working with *Bacillus subtilis* and Dorner and Thoni (14) using cultures of propionic acid bacteria made similar observations. Elliker and Frazier (15) observed that the percentage survival of *E. coli* after heat treatment increased as the temperature at which the culture was grown was increased. Theophilus (40) and Theophilus and Hammer (41), working with spore-forming bacteria important in the spoilage of evaporated milk, found that maximum heat resistance occurred at the optimum temperature of growth, while growth of cultures above and below the optimum resulted in decreased thermal tolerance. The desirability of low storage temperatures for raw milk to en-

hance the effectiveness of thermal processes was emphasized. Earlier Sherman, Stark, and Stark (35) applied the same reasoning to the case of raw milk before pasteurization. Much of the literature concerning the effect of temperature of incubation on heat resistance is confusing because little consideration has been given to the stage of growth of cultures which were used.

Studies were undertaken to determine the effect of temperature of incubation on the level of heat resistance and on the z values of the thermal death-time curves of culture 02. Temperatures of incubation were 20°, 30°, and 37° C. From an examination of growth curves of this culture (figure 5) it was evident that the same relative stage of growth for cultures incubated at 20°, 30°, and 37° C. was reached at approximately 40, 28, and 24 hours, respectively. Therefore, to keep the stage of growth constant when the effect of temperature of growth was studied, the incubation periods at 20°, 30°, and 37° C. were 40, 28, and 24 hours, respectively.

The results shown in figure 6 were obtained from a direct comparison of the effect of these three growth temperatures on heat resistance. The pasteurization curve is included for comparison. The only difference in manipulation of the cultures was in the temperature and period of incubation. It is evident that the culture incubated at 20° C. was much less resistant than those grown at 30° or 37° C. The z values for the curves are 9.7, 11.4, and 11.5 for the cultures incubated at 20°, 30°, and 37° C., respectively. It may be observed that the level of heat resistance which was possessed by the culture incubated at 30° C. was similar to that observed in previous trials for the majority of the cultures which were incubated at 37° C. for 24 hours.

Since temperature of growth is a major factor influencing heat resistance, it was thought that perhaps tem-

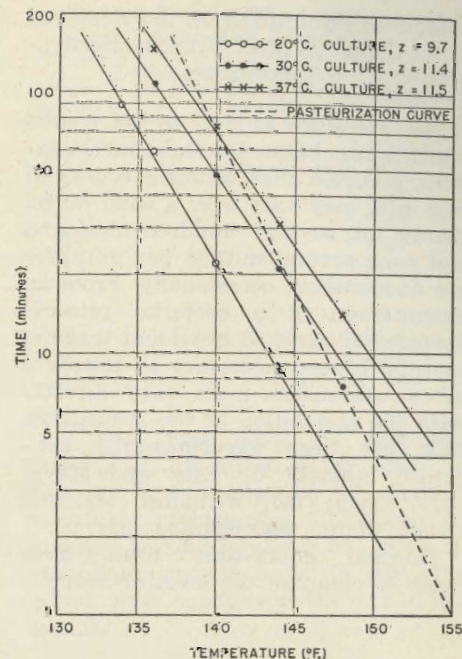


FIG. 6. Thermal death-time curves: Culture 02 after growth in skim milk for 40, 28, and 24 hours at 20°, 30°, and 37° C., respectively (population approximately 50,000 per milliliter)

perature fluctuations during incubation might be responsible for some of the variations in heat resistance which were observed in previous trials. To obtain information in that regard, trials were made using constant-temperature water baths for incubation. The temperatures of the baths were controlled to within $\pm 0.1^\circ$ C. of the temperature which was desired. Incubation was for 24 hours at 34.5°, 35.5°, 36.5°, 37.5°, and 38.5° C. prior to heat treatment at 140° F. While some differences in heat resistance were observed, it did not seem apparent that slight fluctuations in temperature of incubation were responsible for the extreme variations in heat resistance which were observed in previous trials.

Effects of Population on the Survival of Cultures after Various Heat Treatments

The literature as well as the results which were obtained in these investigations revealed that coliform counts of raw milk may vary over a wide range. Counts of several hundred thousand and even several million per milliliter are encountered occasionally. From an examination of a bacterial rate-of-destruction curve, it is evident that the thermal process necessary to destroy a given suspension must vary directly with the population of the suspension. This has been experimentally confirmed, notably by Esty and Meyer (17), Estey (16), Williams (48), and Gross, Vinton, and Stumbo (22).

Thermal death-time curves were made to compare the level of heat re-

sistance possessed by a culture when the population of suspensions differed widely. Suspensions were made of culture 02 having initial populations of approximately 5,000,000 per milliliter. Suspensions with populations of approximately 50,000 per milliliter were included for comparison, although in these instances only one temperature, 140° F., was used for the exposures. Figures 7 and 8 show two curves which were obtained using suspensions of approximately 5,000,000 per milliliter. The z values were 11.7 and 13.0. The organisms in the suspension containing 50,000 per milliliter used in connection with trial 1 were killed in less than four minutes (the shortest time interval used) at 140° F. while organisms in a similar suspension used in trial 2 were killed in 39 minutes. The organisms in the corresponding suspensions contain-

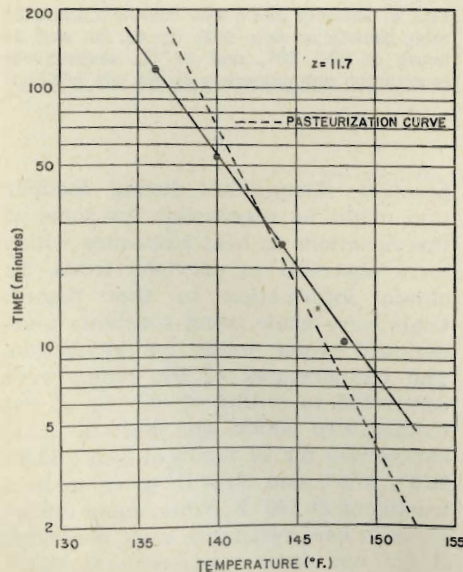


FIG. 7. Thermal death-time curve: Culture 02 after growth in skim milk for 24 hours at 37° C. (Trial 4, population approximately 5,000,000 per milliliter)

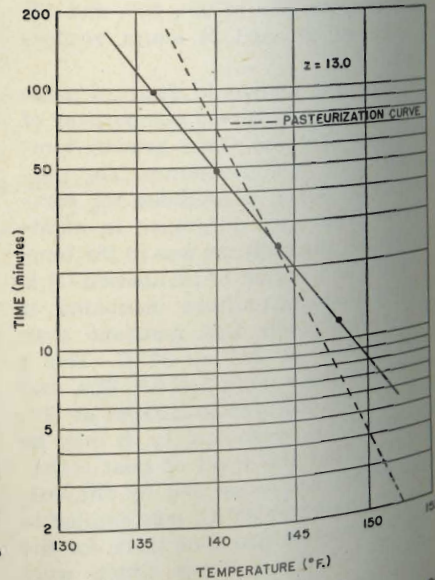


FIG. 8. Thermal death-time curve: Culture 02 after growth in skim milk for 24 hours at 37° C. (Trial 4, population approximately 5,000,000 per milliliter)

ing 5,000,000 per milliliter were killed in 55 minutes and 50 minutes, respectively, at 140° F. Thus, an increase in thermal death-time of approximately 10 minutes at 140° F. occurred as a result of increasing the population from 50,000 to 5,000,000 per milliliter.

Delayed Growth after Heat Treatment

To indicate the importance of incubating a culture for a sufficient period of time before concluding that it was killed by heat, detailed data from one of the experiments are shown in table 5. These data represent many which were obtained. It may be observed that if the tubes had been read at the end of three days of incubation, all tubes would have been recorded as showing no growth. Two tubes did not show growth until after four days, three after five days, two after seven days, and

Table 5. Delayed Growth of Culture 02 After Heat Treatment

Tube number	Period of incubation required for culture to show growth after exposure for 65 minutes at 138° F.
1	7 days
2	no growth in 10 days
3	4 days
4	7 days
5	5 days
6	4 days
7	5 days
8	9 days
9	no growth in 10 days
10	5 days

one after nine days. As mentioned previously, all tubes in the course of this work were read at ten days. In a number of instances tubes were incubated beyond ten days but in no instance did growth occur in any tubes which were negative at the end of that period.

DISCUSSION

Milk may be pasteurized in accordance with the laws and regulations of most cities and states by two processes, the low-temperature, holding method and the high-temperature, short-time process. The commonly prescribed minimum temperature and time relationship for the former is 143° F. for 30 minutes and for the latter is 160° F. for 15 seconds. The increased use of the high-temperature, short-time process for pasteurizing milk and the recent application of this process, modified somewhat, to ice cream mix and milk for cheese manufacture has resulted in certain problems relative to bacterial destruction. One of these originated from observations that bacterial destruction by the high-temperature, short-time process of milk pasteurization was not as great as that obtained by the low-temperature, holding method.

It may be noted frequently in various discussions of this problem that the reasons for differences in effectiveness of the two processes in killing bacteria in milk are unknown. Ball (4) was probably the first to put forth a reasonable scientific explanation. This was based on the relation of bacterial thermal death-time curves to the pasteurization curve. He was of the opinion that thermal death-time curves which have levels of position near that of the pasteurization curve would have z values greater than that of the latter. Therefore, the thermal death-time curve of a culture may be located in a position relative to the pasteurization curve that would indicate destruction by an exposure similar to the low-temperature, holding method but survival by an exposure similar to the high-temperature, short-time process. This appeared to be a logical explanation.

Ball pointed out the lack of data pertaining to the thermal destruction of thermoduric bacteria in milk with which to prove his contention.

The data which were obtained in the present investigation using *Escherichia coli* provide supporting evidence for the explanation advanced by Ball. It is recognized that the coliform bacteria are not considered thermoduric as a group. Nevertheless, the data obtained from a study of their thermal death-time relationships, particularly the *z* values of thermal death-time curves, indicate what might be expected among non-spore-forming bacteria possessing a level of heat resistance near that of the pasteurization curve. The *z* values which were obtained ranged from 9.7 to 13. It is significant that all *z* values were greater by a considerable margin than the value for the pasteurization curve of 8.25. This would indicate that it would be possible for certain bacteria to be destroyed by the low-temperature, holding method, yet survive the high-temperature, short-time process. It should be mentioned that such instances would be likely to occur only with organisms which would show a thermal death-time at 143° F. of a few minutes less than 30 minutes. The exact time would depend on the *z* values of their thermal death-time curves. If a certain proportion of the bacterial population in a raw milk supply was of such a nature, then it is conceivable that differences in bacterial destruction by the two pasteurization processes would occur. If the bacterial population in a raw milk supply consisted of relatively heat-sensitive types characterized by thermal death-time curves considerably below the pasteurization curve, it might be expected that the two processes would appear to be equal with respect to bacterial destruction. The latter situation then, would provide a logical explanation for the instances which have been observed when differences in bacterial destruc-

tion by the two pasteurization processes were not evident.

It should be mentioned at this point that in the commercial application of the two pasteurization processes the heat treatment given would be greater than the minimum time-temperature relationships mentioned above might imply. A considerable period of time elapses while the product is raised to the holding temperature as well as during the cooling process after the holding period. These periods of time may be converted to exposure time at the respective holding temperature, and therefore, constitute holding time in addition to the minimum which may be specified. The magnitude of this additional time factor will, of course, depend upon the type of pasteurizing unit and the effect that other factors might have in determining the time consumed in raising or cooling the product to or from the holding temperature. Some of these factors are: temperature of heating or cooling water, temperature of milk before heat is applied, efficiency of heat transfer, size of unit and speed of milk flow (dependent upon pump speed, pipe diameter, and space volume between plates). In the destruction of microorganisms by heat, this additional time factor may have considerable lethal effect. This is an additional factor to be considered in explaining differences in bacterial destruction as observed through use of different pasteurization processes and equipment differing in capacity and design.

While it is relatively simple to measure heating and cooling equivalent periods in experimental laboratory equipment and in vat-type commercial pasteurizers, it is quite a different matter in the case of the common commercial high-temperature, short-time units. This is indicated by certain of the factors mentioned previously which must be considered in any measurements of this nature.

The temperatures at which *Escherichia coli* (culture 02) was grown were shown to influence markedly its thermal death-time at various temperatures. The effect was great enough to show that the culture could be killed easily by either pasteurization process after growth at 20° C. However, when the culture was grown at higher temperatures (30° to 37° C.) its heat resistance was increased significantly so that probability of its destruction, especially by the high-temperature, short-time process, would be extremely doubtful.

Similar observations were made with respect to population. An increase in thermal death-time of approximately ten minutes at 140° F. was observed when the population of the suspension was increased from 50,000 to 5,000,000 per milliliter. The data obtained indicated that suspensions with intermediate populations would show thermal death-times accordingly.

The *z* values which were observed varied considerably, not only in studies involving different conditions of culture manipulation, but even in repeated trials under similar conditions. Lack of refinement in technique may have

been partly responsible for variations observed. The *z* value of 9.7 for the culture grown at 20° C. was the lowest which was obtained for all thermal death-time curves which were plotted. The values for the cultures grown at 30° and 37° C. were 11.4 and 11.5, respectively. This might indicate that as the temperature at which the culture was grown was increased, the *z* value of its thermal death-time curve would be increased. In this connection, the data of Slatter and Halvorson (37) indicated that *z* values were greatest among the most heat-resistant *Lactobacillus* species which were studied. Further experimentation is necessary before a definite conclusion can be reached.

At present, interest is evident in the efficiency of high-temperature, short-time processes in destroying bacteria (pathogens and nonpathogens). It might be well to concentrate more effort on the characterization of thermal death-time curves of microorganisms important to the dairy industry. With the aid of such knowledge, it would be simple to apply the heat treatment, including a sufficient safety factor, to accomplish the desired result.

CONCLUSIONS

1. A wide variety of coliform bacteria may be expected to be present in dairy products. Eleven of the possible sixteen "Imvic" types were observed among the 139 cultures studied in these investigations. Of the 15 types described by Wilson (49), 13 were observed.
2. The thermal death-times of coliform bacteria present in dairy products may be expected to vary over a wide range. Those observed in these studies varied from less than five minutes to more than 150 minutes at 135° F.
3. The most heat-resistant coliform cultures resembled *Escherichia coli*.
4. The thermal death-time of a pure culture of *Escherichia coli* was found to vary on repeated trials under similar conditions of culture manipulation. Variations observed were from 39 to 74 minutes at 140° F. Similar variations occurred at other temperatures.
5. The temperature at which a culture of *Escherichia coli* was grown markedly influenced its thermal death-time. The temperature of growth may determine whether or not a culture may survive or be killed by the pasteurization process. Under comparable conditions, the thermal death-time of *Escherichia coli* increased from 22 minutes at

140° F. when it was grown at 20° C. to 74 minutes at 140° F. when grown at 37° C.

6. Thermal death-times at different temperatures were increased by an increase in population.

7. The z values of thermal death-time curves for *Escherichia coli* heated in milk may be expected to vary somewhat on repeated trials when determined by methods similar to those used in these studies. The z values were 10.5 and 10.8 for thermal death-time curves of cultures grown at 37° C. for 16 hours having a population of approximately 50,000 per milliliter when subjected to heat treatment. For those

grown at the same temperature for 24 hours, z values of 10.0 and 11.0 were obtained. In two trials using a culture grown at 37° C. for 24 hours and having a population of 5,000,000 per milliliter at the time of heat treatment, the z values were 11.7 and 13.0

8. The data obtained in these studies showed that the z values of thermal death-time curves for coliform cultures exceeded the z value of the "pasteurization curve."

9. The data provide evidence in support of the explanation advanced by Ball (4) for the difference in effectiveness of the two common milk pasteurization processes.

LITERATURE CITED

1. BAKER, E. E., and McCLUNG, L. S. Determination of the heat resistance of non-spore-forming bacteria. *Food Res.* 4:21-29. 1939.
2. BALL, C. OLIN. Thermal process time for canned food. *Natl. Res. Council Bul., (U.S.) Pt. I, 7(37)*. 1923.
3. ———. Mathematical solution of problems in thermal processing of canned food. *Calif. Univ., Pubs., Pub. Health* 1(2):15-245. 1928.
4. ———. Short time pasteurization of milk. *Indus. and Engin. Chem.* 35:71-84. 1943.
5. BARRITT, MAXWELL M. The intensification of the Voges-Proskauer reaction by the addition of a-naphthol. *Jour. Path. and Bact.* 42:441-454. 1936.
6. BASELT, F. C. Information on the use of the "Z" symbol. Cited by C. Olin Ball. *Indus. and Engin. Chem.* 35:76. 1943; also personal communication. 1950.
7. BEAMER, PARKER R., and TANNER, FRED W. Resistance of non-spore-forming bacteria to heat. *Zentbl. f. Bakt., Parasitenk. u. Infektionskrank. Abt. II.*, 100:81-98. 1939.
8. BERGEY, DAVID H., BREED, ROBERT S., MURRAY, E. G. D., and HITCHENS, A. PARKER. *Bergey's Manual of Determinative Bacteriology*. Ed. 5, 1529 pp. The Williams and Wilkins Company, Baltimore. 1939.
9. BIGELOW, W. D. The logarithmic nature of thermal death time curves. *Jour. Infect. Dis.* 29:528-536. 1921.
10. BIGELOW, W. D., BOHART, G. S., RICHARDSON, A. C., and BALL, C. O. Heat penetration in processing canned foods. *Natl. Canners Assoc. Bul.* 16-L. 1920. (Original not seen.) Cited by C. Olin Ball, *Calif. Univ., Pubs., Pub. Health* 1(2):15-245. 1928.
11. CHICK, HARRIETTE. An investigation of the laws of disinfection. *Jour. Hyg. (London)* 8:92-126. 1908.
12. CLARK, WILLIAM MANSFIELD, and LUBS, HERBERT A. The differentiation of the colon-aerogenes family by the use of indicators. *Jour. Infect. Dis.* 17:160-173. 1915.
13. COMMITTEE ON BACTERIOLOGICAL TECHNIC OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS. *Manual of Methods for Pure Culture Study of Bacteria*. Biotech. Publications. Geneva, New York. 1946.
14. DORNER, W., and THONI, MARG. Zur Biologie der Propionsaurebakterien. *Landw. Jahrb. der Schweiz.* 50:889-898. 1936.
15. ELLIKER, PAUL R., and FRAZIER, W. C. Influence of time and temperature on heat resistance of *Escherichia coli*. *Jour. Bact.* 36:83-98. 1938.

16. ESTY, J. RUSSELL. The heat resistance of *Bacillus botulinus* spores. Amer. Jour. Pub. Health 13:108-113. 1923.
17. ——— and MEYER, K. E. The heat resistance of the spores of *B. botulinus* and allied anaerobes. Jour. Infect. Dis. 31:650-663. 1922.
18. GILCREAS, F. W., and O'BRIEN, J. E. Studies of thermal death ranges of *Bacterium coli* in milk. N. Y. State Assoc. of Milk Sanitarians, 22nd Ann. Rpt. 1948:9-21. 1949.
19. GILLESPIE, T. G. The heat resistance of the spores of thermophilic bacteria. Fruit and Veg. Preserv. Res. Sta., Campden, Ann Rpt. 40, 1948. (Original not seen.) Cited by J. M. Reed, C. W. Bohrer, and E. J. Cameron. Food Res. 16:383-408. 1951.
20. GROSS, C. E., and SCHAUB, D. J. Evaluation of the lethality of pasteurization processes used in the meat packing industry. Inst. Food Technol., Proc. 1945:139-145. 1945.
21. ——— and VINTON, C. Thermal death time of a strain of *Staphylococcus* in meat. Food Res. 12:188-202. 1947.
22. ——— VINTON, C., and STUMBO, C. R. Bacteriological studies relating to thermal processing of canned meats. VI. Thermal death time curve for spores of test putrefactive anaerobe in meat. Food Res. 11:411-418. 1945.
23. HALVORSON, H. O. Information on time conversions. Personal communication. 1948.
24. HOLLAND, R. F., and DAHLBERG, A. C. The effect of the time and temperature of pasteurization upon some of the properties and constituents of milk. N. Y. (Geneva) Agr. Expt. Sta., Tech. Bul. 254. 1940.
25. KOSER, S. A. Utilization of the salts of organic acids by the coliform aerogenes group. Jour. Bact. 3:493-520. 1923.
26. KRONIG, B., and PAUL, TH. Die chemische Grundlager der Lehre von der Giftwirkung und Desinfection. Ztschr. f. Hyg. u. Infektionskrank. 25:1-112. 1897.
27. OLDENBUSH, CAROLYN, FROFISHER, MARTIN, SR., and SKRADER, J. H. Thermal death points of pathogenic bacteria in cream and ice cream. Amer. Jour. Pub. Health 20:615-618. 1930.
28. OLSON, J. C., JR. A study of certain factors pertaining to the heat resistance of coliform bacteria in milk. Ph.D. thesis, University of Minnesota. Filed in Library, Univ. of Minn. 1948.
29. PARK, WILLIAM H. Thermal death points of pathogenic bacteria in milk. Amer. Jour. Pub. Health. 17:36-47. 1927.

30. ——— Thermal death time of streptococci. Amer. Jour. Pub. Health 18:710-714. 1928.
31. PARK, S. E., GRAHAM, ROBERT, PRUCHA, M. J., and BRANNON, J. M. Pasteurization of milk artificially infected with two strains of *Brucella suis*. Jour. Bact. 24:461-471. 1932.
32. PARR, LELAND W. Coliform bacteria. Bact. Rev. 3:1-48. 1939.
33. RAHN, OTTO. Physical methods of sterilization of microorganisms. Bact. Rev. 9:1-47. 1945.
34. SCHMIDT, C. F. Information on use of the symbol "D." Personal communication cited by J. M. Reed, C. W. Bohrer, and E. J. Cameron. Food Res. 16:383-408. 1951.
35. SHERMAN, J. M., STARK, C. N., STARK, PAULINE. An unappreciated but important factor in the pasteurization of milk. Jour. Dairy Sci. 12:385-393. 1929.
36. SLATTER, W. L. The heat resistance of lactobacilli found in American cheddar cheese. Ph.D. thesis. University of Minnesota. Filed in Library, Univ. of Minn. 1946.
37. ——— and HALVORSON, H. O. The heat resistance of lactobacilli found in American cheddar cheese. Jour. Dairy Sci. 30:231-243. 1947.
38. SPECK, M. L. The resistance of *Micrococcus freudenreichii* in laboratory high-temperature--short-time pasteurization of milk and ice cream mix. Jour. Dairy Sci. 30:975-981. 1947.
39. STUMBO, C. R., MURPHY, J. R., and COCHRAN, JEANNE. Nature of thermal death time curves for *P. A. 3679* and *Clostridium botulinum*. Food Technol. 4:321-325. 1950.
40. THEOPHILUS, D. R. Influence of growth temperature on the thermal resistance of some aerobic spore-forming bacteria from evaporated milk. B. W. Hammer Panegyric, pp. 161-176. Collegiate Press, Inc. Ames, Iowa. 1937.
41. ——— and HAMMER, B. W. Influence of growth temperature on the thermal resistance of some bacteria from evaporated milk. Iowa Agr. Expt. Sta., Res. Bul. 244. 1933.
42. TITSLER, RALPH P., and SANDHOLZER, LESLIE A. The use of semi-solid agar for the detection of bacterial motility. Jour. Bact. 31:575-580. 1936.
43. ——— and SANDHOLZER, LESLIE A. Advantages of peptone iron agar for the routine detection of hydrogen sulfide production. Amer. Jour. Pub. Health 27:1246-1242. 1937.

44. VAUGHN, REESE, MITCHELL, N. B., and LEVINE, MAX. The Voges-Proskauer and methyl red reactions in the coli-aerogenes group. Amer. Waterworks Assoc. Jour. **31**:993-1001. 1939.
 45. WATKINS, J. H., and WINSLOW, C. E. A. Factors determining the rate of mortality of bacteria exposed to alkalinity and heat. Jour. Bact. **24**:243-265. 1932.
 46. WEIL, RICHARD. Zur Biologie der Milzbrandbacillen. Arch. f. Hyg. **35**:355-408. 1899. (Original not seen.) Cited by O. B. Williams. Jour. Infect. Dis. **44**:421-465. 1929.
 47. WEISS, H. The heat resistance of spores with special reference to the spores of *B. botulinus*. Jour. Infect. Dis. **28**:70-92. 1921.
 48. WILLIAMS, O. B. Heat resistance of bacterial spores. Jour. Infect. Dis. **44**:421-465. 1929.
 49. WILSON, G. S. The bacteriological grading of milk. Med. Res. Council (London), Special Rpt. Ser. No. 206, 1935.
-

